Pestic. Sci. 1996, **47**, 355–362

Degradation Studies of the Non-lethal Bird Repellent, Methyl Anthranilate

Eugeny V. Aronov,[a]*† & Larry Clark[a,b]

[a] Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA
[b] United States Department of Agriculture, National Wildlife Research Center, Fort Collins, CO 80523, USA

(Received 6 June 1995; revised version received 30 October 1995; accepted 13 March 1996)

Abstract: Methyl anthranilate (MA), a food grade flavor and fragrance additive, has been reported to be an effective non-lethal bird repellent in a variety of situations. Despite the experimental success of MA, field studies have yielded widely differing levels of efficacy. Diminished efficacy in some field trials probably results from the failure of specific formulations to retain or protect the active ingredient under natural conditions. Therefore, a clearer understanding of the physical and chemical factors affecting the stability of MA is needed. We undertook a series of laboratory studies on hydrolysis, photolysis and microbial degradation of MA, the results of which could be useful in the development of appropriate formulation strategies and residue analyses. We found the *n*-octanol : water partition coefficient, (*P*) to be 84. MA is not subject to hydrolysis at 25°C in phosphate buffer media at pH 5·0, 7·0 and 9·0. MA slowly photodegrades under simulated UV 'sunlight'. Forty-four percent of MA is lost after 432 h illuminance at 1·25 mW cm^{-2}, which is equivalent to approximately 1200 h natural sunlight (40°N, noontime, June). Kinetic data indicate that the initial step of photolysis, subsequent to excitation, is a second-order reaction with respect to MA. A major photodegradation product appeared in an amount of about 10% of the mass balance and was determined to be an oxidized trimer of MA. MA is primarily affected by aerobic microbial degradation. For a 12 : 12 h light : dark, under laboratory illumination, 12% of water-solubilized material can be lost in the first seven days. Losses were 30% and 42% at 16 and 27 days, respectively. Under conditions of optimal bacterial growth (warmth and darkness) loss of MA was 22% at nine days and 100% by 20 days. The susceptibility of MA to microbial degradation is promising for the prospects of developing formulated, environmentally safe, bird repellents.

Key words: animal damage control, bird repellent, methyl anthranilate, photolysis, hydrolysis, partition coefficient

1 INTRODUCTION

Methyl anthranilate (MA) is a naturally occurring compound found in a variety of flowers and fruits.[1–3] MA has also been found in fungi, ants,[4] fish and birds (Hapraz, S. pers. comm.). To humans, MA has a grape or floral odor, hence its widespread use as a flavor and fragrance additive.[5] Despite its pleasant hedonic characteristics to humans and other mammals, MA and its derivatives are aversive to birds[6] and have been used as non-lethal bird repellents in the field.[7–11]

Birds can cause serious damage to a wide range of agricultural and horticultural commodities.[8,9,11–13] Conversely, many of man's activities can negatively impact upon protected bird populations.[12,14,15] In both cases, the control of movement of bird populations and restriction of their use of a resource is needed.

The recent US Environmental Protection Agency's registration of formulations containing MA (EPA registration numbers 58035-6, 58035-7, 58035-9) for use in

* To whom all correspondence should be addressed.
† Current address: Schering-Plough Research Institute, K-15 B211/2800 2015 Galloping Hill Rd., Kenilworth, NJ 07033-0539, USA.

bird control holds promise for incorporating this compound into a non-lethal integrated wildlife management plan. Yet field studies have yielded widely differing levels of efficacy.[16,17] Diminished efficacy in some field trials is not a failure of the active ingredient, because laboratory and pen studies have uniformly demonstrated the efficacy of MA.[18–21] Rather, diminished efficacy results from the failure of specific formulations to retain or protect the active ingredient under natural conditions. Achieving the appropriate matrix to carry and retain MA is a critical component in the success of this non-lethal wildlife management strategy. Toward this end, a clearer understanding of the physical factors affecting the stability of MA is needed. We undertook a series of laboratory studies on hydrolysis, photolysis and microbial degradation of MA, the results of which could be useful in the development of appropriate formulation strategies and residue analyses.

2 EXPERIMENTAL METHODS

2.1 Instrumentation

High performance liquid chromatography (HPLC) was used to assay for MA. The system consisted of a Rainin HPXL solvent delivery system, Dynamax AI-2 autosampler equipped with a 20-μl fixed sample loop and a Dynamax UV-M ultraviolet detector. A Hewlett Packard 3390A recording integrator was employed for quantitative analyses.

The reactor for the study of MA photodegradation included a bank of three RPR-3000A lamps (maximum emission at 300 nm, total power = 8 W per lamp) and a quartz rectangular reaction vessel (18 × 5 × 5 cm) with a sterilized rubber sealing stopper equipped with sampling port and nitrogen inlet. Light intensity inside the reactor was measured prior to the experiments using a Spectroline DRC-100X digital radiometer equipped with a DIX-300 sensor (maximum sensitivity at 300 nm). The same device was used to measure natural sunlight intensity at noon, 25 June in Philadelphia, PA (approximately 39°N).

Spectral data were obtained using a Brucker MSL-400 (400 MHz) NMR spectrometer, Finnegan GC/MS spectrometer equipped with a Data General Nova computer system, a Shimadzu IR-435 infrared spectrophotometer and a Gilford 2600 ultraviolet spectrophotometer.

2.2 Semi-empirical quantum mechanical calculations

These calculations were performed using MOPAC 6.3 software in order to justify the choice of an artificial light source for photolysis studies. Geometry of the ground state of the MA molecule has been optimized with PM3 parameter set (keywords: SINGLET, EF GNORM = 0·01), followed by the calculation of excitation energies by Multielectron Configurational Interactions (MECI) technique (keywords: 1SCF, SINGLET, MECI, C.I. = 3).

2.3 Chemicals and solvents

Methyl anthranilate (CAS 134-20-3) was purchased from Fluka Chemical Company. The sample purity was specified as >98% GC. All test concentrations were based on the total compound, i.e. not corrected for sample purity. All solvents were of HPLC grade (Aldrich Chemical Co.) and were used without additional purification.

Thin layer chromatography (TLC) of MA photodegradation products was performed using Kieselgel RP-18 (reversed phase) plates (E. Merck) with acetonitrile + water (8:2 by volume) as developing system. TLC chromatograms were visualized under both visible and UV light.

2.4 Quantitative determination of MA

A general HPLC analytical method was used to analyze the MA solutions. The column was a Zorbax ODS 4.6 × 250 mm with 50-mm guard column. The mobile phase was acetonitrile + water (1:1 by volume) at a flow of 1 ml min^{-1}, with detection being performed at either 330 or 280 nm, depending on sample concentration. Retention time of MA under these conditions is approximately 7 min. A calibration of detector response at both wavelengths was carried out prior to analyses. Concentration range in which detector response was a linear function of concentration was 5–200 mg $litre^{-1}$ and 100–5000 mg $litre^{-1}$ for detection wavelengths of 330 nm and 280 nm respectively.

2.5 Octanol-water partition

The purpose of this study was to determine octanol-water partition coefficient of MA. Two solutions of MA were prepared: 100 and 50 μl $litre^{-1}$. Aliquots (2 ml) of each MA solution were placed into 10-ml test tubes. *n*-Octanol (2 ml) was added to each of the test tubes, and the resultant mixtures were sonicated by ultrasound for 6 h. After sonication, emulsions were centrifuged at 1000*g* for 10 min, and MA concentrations in both octanol and water layers were determined by analytical HPLC. The partition coefficient, P was calculated as the average ratio of MA peak area in octanol over MA peak area in water.

2.6 Hydrolysis

In this study we were interested in the susceptibility of MA to hydrolysis. A solution of MA was prepared as

follows: 3 ml of MA was added to 300 ml of deionized water which had been sterilized *via* autoclave. The mixture was stirred for 12 h at 24°C, after which the stirrer was turned off in order to allow undissolved particles of MA to sink to the bottom.

Starting solutions were prepared by taking three 50-ml aliquots of the saturated MA solution *via* sterile syringe. These aliquots were injected into three sterile solutions (50 ml of each) of 0·2 M phosphate buffer having pH 5·0, 7·0 and 9·0, respectively. Solutions were stored at 24·5°C in the dark. Samples of each solution were taken for HPLC analysis at the moment of preparation and then every three to four days, thereafter.

2.7 Kinetic study of MA photodegradation

The object of this study was to determine the rate of photodegradation of MA under quantified irradiance conditions. The choice of light source for the study and its appropriateness are discussed below (see Section 3.3.1). An aqueous solution of MA (150 ml) was prepared as described above and mixed with 150 ml of a sterile phosphate buffer (0·2 M, pH 7·0). The MA concentration obtained was found to be 7·04 mM (1077 mg litre^{-1}). The mixture was placed into a sterilized photoreaction vessel containing a magnetic stirring bar. The headspace above the solution was replaced with nitrogen. The reaction mixture was continuously stirred and irradiated by UV light with maximum emission at 300 nm and intensity of 1·25 mW cm^{-2} (260 mW per reaction mixture). Photolysis was monitored by HPLC with aliquots taken every 4 h on the first day and once a day thereafter. Analysis for photodecomposition products was performed using a Zorbax ODS (4·6 × 250 mm) column with a 50-mm guard column in linear gradient of acetonitrile in water from 50% to 80%, for 20 min with subsequent holding of the mobile phase composition for 10 min. Outcoming compounds were detected by high speed wavelength scanning technique at 2-nm intervals for the ranges of 370 to 800 nm, and 200 to 365 nm.

2.8 Preparation and isolation of MA photodegradation products

The goal of this study was to obtain photodecomposition products of MA in quantities sufficient for structure determination and calculation of the mass balance. The procedure described in Section 2.6 was repeated with a starting concentration of MA of 16·23 nM (2483 mg litre^{-1}). Irradiation was terminated after 384 h when the concentration of MA left was 7·97 mM (1220 mg litre^{-1}), i.e. 49% of the MA at the beginning of the experiment. A brown precipitate, formed in the reaction, was separated by centrifugation, washed with water and dried under vacuum. The precipitate obtained (132 mg, or 32·6% of the mass of the reacted MA) was dissolved in 200 μl of chloroform and separated portion-wise using preparative TLC plates (20 × 20 cm). Three colored bands with R_f 0·5, (band 1), 0·7 (band 2) and 0·8 (band 3) were scraped out and extracted with chloroform. Extracts were filtered, evaporated, dried under vacuum (oil pump, 30 min) and weighed. After the removal of the coloured bands, the rest of the plate coating material was extracted with chloroform. The extract was filtered, evaporated and the residue was dried under vacuum and weighed. Weights of the fractions corresponding to bands 1, 2, 3 and the rest of the plate were 32 mg, 17 mg, 24 mg and 59 mg, respectively. The major product (MAPP, band 1) constituting more than 10% of the reacted MA mass, was purified portionwise by HPLC (Zorbax ODS 4.6 × 250 mm, acetonitrile + water (65:35 by volume), 1 ml min^{-1}, detection at 300 nm, 5 mg of MAPP in 500 μl of acetonitrile per injection). Homogeniety of the purified MAPP was confirmed by analytical HPLC under the conditions described above (see Section 2.7). The following are spectral data of the purified MAPP. MS (solid probe; *m/z*, rel. intensity %): 467 (M + 2, 12), 466 (M + 1, 9), 465 (M, 50), 463 (M − 2, 100), 430 (29), 404 (71), 372 (73), 340 (48), 328 (35); IR 10 g litre^{-1} in chloroform, cm^{-1}): 3518, 3400, 3020, 2965, 1690, 1625, 1595, 1570, 1497, 1465, 1446, 1305, 1251, 1200, 1170, 1115; UV (solution in acetonitrile-0·2 M phosphate buffer (pH 6·8), λ_{max} nm): 215, 249, 273, 384; [^{1}H]NMR (deuterochloroform, δ ppm): 3·09 (s, 3H), 3·95 (s, 3H), 3·97 (s, 3H), 6·51 (s, 1H), 7·10 (d, J = 8·2 Hz, 1H), 7·11 (ddd, $J_1 = J_2 = 7·6$ Hz, $J_3 = 1·0$ Hz, 1H), 7·17 (ddd, $J_1 = J_2 = 7·6$ Hz, $J_3 = 1·3$ Hz, 1H), 7·46 (ddd, $J_1 = J_2 = 7·8$ Hz, $J_3 = 1·6$ Hz, 1H), 7·58 (ddd, $J_1 = J_2 = 7·2$ Hz, $J_3 = 1·6$ Hz, 1H), 7·84 (dd, $J_1 = 8·2$ Hz, $J_2 = 1·0$ Hz, 1H), 8·00 (dd, $J_1 = 8·1$ Hz, $J_2 = 1·6$ Hz, 1H), 8·08 (dd, $J_1 = 7·9$ Hz, $J_2 = 1·6$ Hz, 1H), 10·1–10·7 (broad s, 1H), 10·7–11·5 (broad s, 1H), 11·73 (broad s, 1H); [^{13}C] (deuterochloroform, δ ppm): 50·9 (q, 1C), 52·5 (q, 2C), 98·8 (s, 1C), 99·5 (d, 1C), 119·5 (d, 1C), 120·1 (s, 1C), 120·3 (s, 1C), 123·4 (d, 1C), 123·5 (d, 1C), 131·5 (d, 1C), 132·0 (d, 1C), 133·2 (d, 1C), 133·7 (d, 1C), 140·3 (s, 1C), 141·7 (s, 1C), 142·0 (s, 1C), 149·5 (s, 1C), 158·9 (s, 1C), 166·6 (s, 1C), 167·0 (s, 1C), 167·2 (s, 1C), 180·5 (s, 1C).

2.9 Microbial degradation

Our previous observations indicated a disappearance of MA from aqueous solutions under nonsterile conditions. This led to the hypothesis that MA might be subject to microbial attack. The purpose of this study was to evaluate the possibility of microbial degradation of MA. Identification of possible degradation products was not attempted. Two solutions of approximately 50 mg litre^{-1} of MA in dechlorinated, charcoal-filtered

water were prepared and placed in 10-litre test vessels. Vessels were stored open to natural inoculation in a water bath maintained at 13–15°C for seven days prior to initiation of the test. After seven days one tank received 1 g of sodium azide, a metabolic poison, resulting in a final concentration of 0·1 g litre^{-1}. The control tank received no further treatment. Samples of 100 ml were drawn, sealed (to prevent further inoculation) and stored at 23°C in sterilized, sealed amber vials. Vials were stored under dark or light : dark (12 : 12 h) conditions. Aliquots from all treatments were analyzed for MA content at intervals of 0, 0·2, 1, 2, 5, 6, 7, 8, 9 and 20 days post-treatment. Volatilization and autooxidation were not controlled because it was found in other experiments that MA is not volatile and because aromatic oxidation products were not found.

3 RESULTS AND DISCUSSION

3.1 Octanol-water partition

The *n*-octanol-water partition coefficient was taken ($n = 4$) as the average ratio of MA concentration in *n*-octanol over MA concentration in water, $P = 84(\pm 0{\cdot}3$ S.E.).

3.2 Hydrolysis

MA was not found to be subject to significant hydrolysis at 25°C in phosphate buffer with pH of 5·0, 7·0 and 9·0. After 38 days in buffered solution, the losses of MA were 0·4%, 3·7% and 3·9% respectively. There was chromatographic evidence of a minor hydrolytic product at pH 9·0, with retention time of this compound being consistent with that of anthranilic acid.

3.3 Kinetic study of photolysis

3.3.1 The choice of artificial light source

The UV spectrum of MA as well as semi-empirical quantum mechanical calculations indicate that energy of the first excited singlet state, S_1, exceeds the energy of the ground state, S_0 by about 3·81 eV. This corresponds to the wavelength of the exciting light of approximately 325 nm (depending upon solvent). The wavelength of 325 nm is present at the almost equal power level in emission spectra of two commercially available lamps, namely RPR 3000A and RPR 3500A (Southern New England Ultraviolet Co.) These artificial light sources are equally appropriate for the $S_1 \leftarrow S_0$ excitation of MA molecules. The 325 nm band is also present in the natural sunlight[22] at the level of 50–100 μW cm^{-2} nm^{-1}. Emission spectra of both artificial light sources and the spectrum of the natural sunlight are shown in Fig. 1.

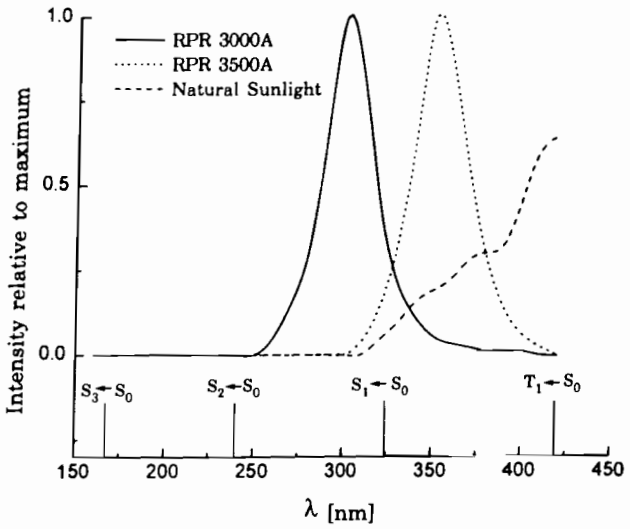

Fig. 1. Emission spectra of two artificial light sources (Southern New England Ultraviolet Co.) and natural sunlight[22] with a diagram of electronic excitation energies for MA molecule.

The calculated energy of T_1 state of MA is 2·90 eV, which corresponds to the wavelength of 420 nm of the exciting light. This wavelength is not present in the spectrum of the artificial source chosen for our study (RPR 3000A), while in natural sunlight its intensity is about 730 μW cm^{-2} nm^{-1} [22] (Fig. 1). Although T_1 state may play a significant role in photodegradation of MA, the direct $T_1 \leftarrow S_0$ excitation is spin-forbidden and, therefore, should not be of concern when considering a source of artificial light. The spectral difference between the sunlight and the artificial source at wavelengths of visible light may be important for the fate of primary degradation products but should not affect photolysis of MA.

Both computations and spectroscopy indicate that energies of $S_2 \leftarrow S_0$ and $S_3 \leftarrow S_0$ transitions correspond to wavelengths of the exciting light of 240 nm and 160 nm respectively. These bands are present neither in the spectrum of the RPR 3000A lamp nor in the spectrum of the natural sunlight.

3.3.2 Kinetic studies

The starting concentration of MA was 7·04 mM (1077 mg litre^{-1}). After 24 h irradiation, the solution turned from clear to slightly yellow, progressed to a reddish-purple color after 48 h, and finally to a brown color after 72 h. At 76 h a brown precipitate was visible on the bottom of the reaction vessel. Irradiation was terminated after 432 h when the concentration of the remaining MA was 3·94 mM (603 mg litre^{-1}), i.e. 56% of the original solubilized MA. HPLC analysis of the solution indicated very small peaks of degradation products, suggesting that the degradation products were insoluble in water and were primarily located in the precipitate. Structure determination of the major photo-

degradation product as well as a possible mechanism of the process are discussed below (see Section 3.4).

The kinetic data suggest that, prior to the precipitation of photolytic products, the rate law of the reaction is rather complicated. After the precipitation has started and the concentration of degradation products in the solution is nearly constant, photolysis behaves as a reaction of the second order with respect to MA, i.e. the reciprocal concentration of MA is a linear function of time (Fig. 2). The calculated rate constant of this stage of photolysis is $1{\cdot}4 \times 10^{-4}$ litre mmol^{-1} h^{-1}. Because natural sunlight (measured at 40°N in June at noon) is about three-fold less intense than the light source used for the study, the rate constant of MA photolysis in the environment should not exceed 5×10^{-5} litre mmol^{-1} h^{-1}. This is a low value for a rate constant. Therefore we do not expect photolysis to play a significant role in the environmental degradation of MA.

3.4 Isolation and identification of major MA photodegradation product (MAPP)

The precipitate obtained from the preparative photolysis was analyzed using both reversed phase TLC and HPLC. Both methods indicated four degradation products. The major MA photodegradation product (MAPP) was isolated by preparative reversed phase TLC and its homogeneity was confirmed by HPLC prior to the structure determination. The UV spectrum of MAPP depends upon the pH of the medium, suggesting that the structure contains both acidic and basic functional groups.

The molecular mass of MAPP appeared to be 465, while the [^{13}C]NMR spectrum contained signals from 24 carbon atoms. There are only two reasonable empirical formulae corresponding to the molecular mass and the number of carbons: $C_{24}H_{23}N_3O_7$ and $C_{24}H_{19}NO_9$. The structure does not contain a primary amino group,

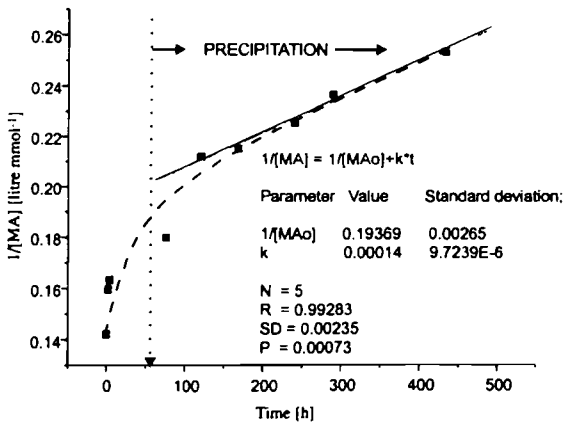

Fig. 2. Kinetics of MA photolysis. After the beginning of precipitation of photolytic products the process behaves as a second-order reaction with rate constant, $k = 1{\cdot}4(\pm 1) \times 10^{-4}$ litre mmol^{-1} h^{-1}.

which was determined by comparison of IR spectra of MA (not shown) and MAPP: the doublet corresponding to NH_2 in MA (3518, 3400 cm^{-1}) disappears in MAPP and a broad absorption band at 3400 cm^{-1} appears instead. Moreover, two non-equivalent H–N protons have been found in the [^{1}H]NMR spectrum of MAPP, i.e. the structure contains at least two secondary amino groups. Thus, $C_{24}H_{23}N_3O_7$ is the only reasonable empirical formula for further consideration, suggesting that the photolysis of MA represents oxidative trimerization where two amino groups form bridges between MA moieties.

Simple calculations show that the unsaturation number (number of rings plus number of double bonds) for the MAPP molecule is 15, which is equal to the tripled unsaturation number of MA. This means that no extra double bounds were formed upon trimerization. Both the [^{13}C] and [^{1}H]NMR spectra contain signals of three methyl groups. Two of these groups have the same chemical shift as an ester methyl group in MA, while the third one is shifted toward the higher field.

Both spectra also show nine aromatic C-H groups. The type of coupling of aromatic protons in [^{1}H]NMR confirms that four of the aromatic C-H groups belong to the (HC)-CH-(CH) type, another four belong to the (CH)-CH-C type and one C-H proton has no C-H neighbors. There is only one possible combination where three differently substituted benzene rings match this result, namely: two 1,2 disubstituted and one pentasubstituted ring. Apparently the 1,2 disubstituted rings are two side MA moieties connected through -NH- bridges to the central one. At this point the question about the position of -NH- bridges in the central ring arises.

Studies of photodecomposition of aromatic amines[23] show that one of the predominant primary processes occurring under irradiation is homolytic breakdown of one of the H-N-H bonds and HN$^{\cdot}$ free radical formation (Fig. 3). This radical is quickly stabilized *via* electron transfer forming either *ortho* or *para* radicals and reacts with another molecule of MA, forming either *ortho* (IIa) or *para* (IIb) dimer. Kinetic data support a bimolecular mechanism in the first step of photolysis. In turn, both dimers (IIa, IIb) form corresponding N$^{\cdot}$ free radicals with consecutive addition of a third MA molecule, giving the same trimer (III). The proposed mechanism explains why photopolymerization is self-terminated at the stage of trimer formation.

The spectral data show that the structure of MAPP is different from that of the trimer (III) because: (i) one of the MAPP methyl groups is not an ester, and its chemical shift in [^{1}H] and [^{13}C]NMR corresponds to either methylamino or methyl ether groups; (ii) the carbon signal at 180·5 ppm in [^{13}C]NMR and the proton signal at 11·73 ppm in [^{1}H]NMR most likely indicate an existence of a free carboxy group; (iii) the empirical formula $C_{24}H_{23}N_3O_7$ requires one more oxygen atom;

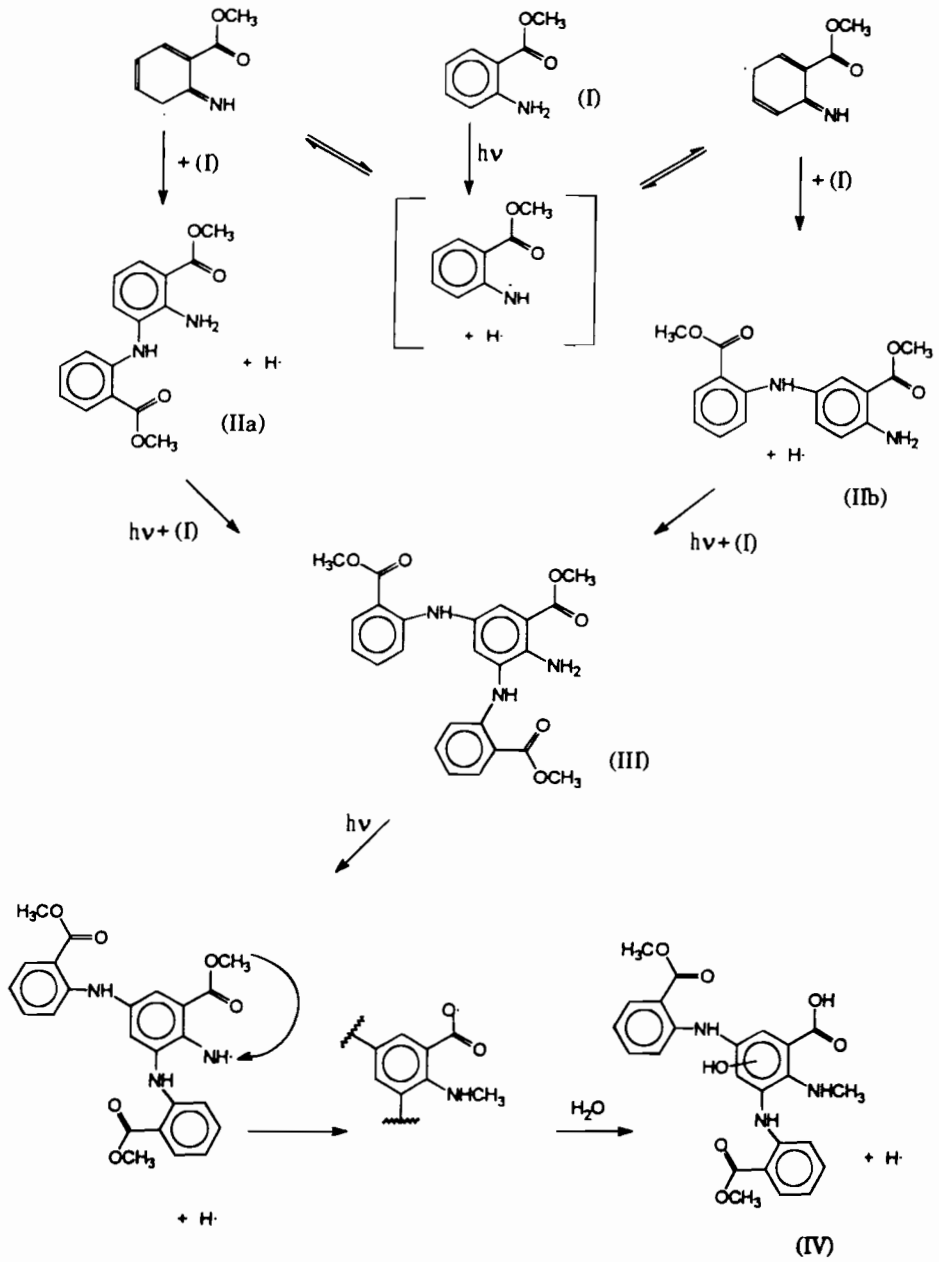

Fig. 3. Suggested mechanism of MA photodegradation under simulated 'sunlight' in oxygen-free aqueous solution.

(iv) both [^{13}H] and [^{1}H]NMR spectra require only one aromatic C-H group, lacking any C-H neighbors.

We assumed that, as long as a primary amino group exists, it keeps forming HN˙ free radicals under irradiation. The trimer (III) forms a free radical having no way of reacting at the *ortho-* or *para*-position. Therefore, it attacks the neighboring methyl ester group causing an intramolecular rearrangement. To comply with the spectral data, the O˙ radical formed needs an addition of a water molecule with a following elimination of an extra hydrogen atom, resulting in compound IV. The mechanism of this step of photolysis remains unknown and can only be speculated.

The resulting structure (IV) is in agreement with all the spectra, and the mechanism proposed explains why it is an ultimate product of MA photolysis. In spite of consistency between the structure (IV) and the spectral data of MAPP we did not find any direct indication of the presence of a hydroxy group and its position. MAPP, for instance, fails a ferric chloride test. This failure could be due to participation of the hydroxy group in an intramolecular hydrogen bond with carbonyl oxygen as well as due to its steric hindrance.

3.5 Microbial degradation

We became interested in microbial action on MA stability following experiments for the determination of the lethal concentration dose for aquatic vertebrates.[24]

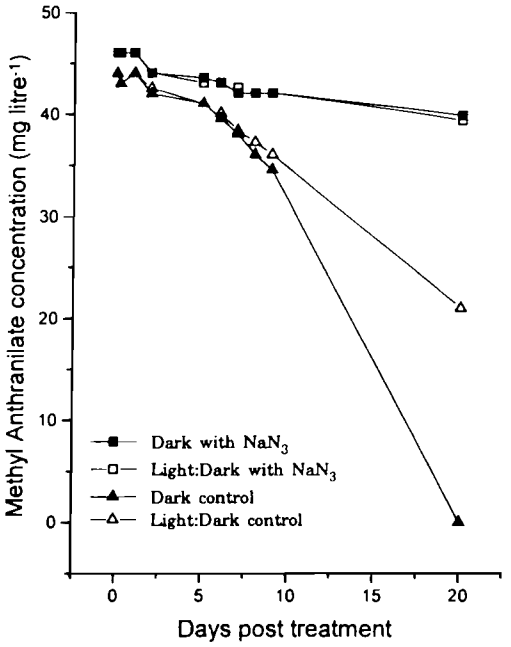

Fig. 4. The concentration of MA as a function of time for the microbial degradation experiments. Open symbols represent samples exposed to laboratory illuminance at a photoperiod of 12 : 12 h light : dark. The closed symbols represent samples kept in continuous darkness.

Under aquaculture conditions, especially those where dead fish were not removed from test tanks promptly, MA disappeared extraordinarily quickly. The following experiments were conducted to replicate laboratory conditions, but in the absence of fish. There was very little loss of MA during the first seven days. This is consistent with our experience that solutions of MA are relatively stable for short periods of time.

After the seven-day inoculation period, there was very little loss of MA in vessels treated with sodium azide, irrespective of ambient light conditions (Fig. 4). Thus, without aerobic bacteria, very little loss of MA occurred. Because the two darkened test vessels showed patterns of degradation similar to those exposed to light, we concluded that loss was not due to photolysis. The observable loss of MA was slightly more than would be expected from hydrolysis alone. Therefore, we attribute the minor loss of MA to anaerobic microbial action (on the order of 10% of the original MA volume over a 20-day period).

Aerobic bacteria have a profound effect on the fate of MA (Fig. 4). In the control test vessels, loss of MA over a 20-day period was on the order of 50% for the vessel maintained on a diurnal light cycle, while there was total loss of MA for the sample held under dark conditions (optimum bacterial growth conditions).

4 CONCLUSIONS

In conclusion, MA is not significantly susceptible to hydrolysis, but is susceptible to photodegradation if exposed to UV radiation. However, photodegradation is not of practical concern for the environmental fate of MA because the rate of aerobic biodegradation of MA is much higher. There was no evidence of aromatic breakdown products in any of the microbial degradation studies. The susceptibility of MA to microbial attack, and its apparent disintegration into non-aromatic degradation products, is promising for the prospects of developing formulated, environmentally safe, bird repellents. However, because MA is easily biodegradable, field efficacy tests should be closely paired with residue analyses to verify the presence of the repellent material.

ACKNOWLEDGEMENTS

This study was broadly supported by a cooperative agreement between the Monell Center and US Department of Agriculture (# 12-34-41-0040).

REFERENCES

1. Opdyke, D. L. J., Monographs on fragrance raw materials. Methyl anthranilate. *Food Cosmet. Toxicol.*, **12** (1975) 935–6.
2. Nelson, R. R., Acree, T. E., Lee, C. Y. & Butts, R. M., Methyl anthranilate as an aroma constituent of American wine. *J. Food Sci.*, **42** (1977) 57–9.
3. Reynolds, A. G., Fuleki, T. & Evans, W. D., Inheritance of methyl anthranilate and total volatile esters in *Vita* species. *Amer. J. Enol. Viticul.*, **33** (1982) 14–19.
4. Duffield, R. M., Wheeler, J. W. & Blum, M. S., Methyl anthranilate: identification and possible function in *Aphaenogaster fulva* and *Xenomyrmex floridanus*. *Fla. Entomol.*, **63** (1980) 203–6.
5. Furia, E. E. & Bellanca, N., *Fenaroli's Handbook of Flavor Ingredients*, Vol. 1. CRC Press, Cleveland, OH, 1975, p. 551.
6. Kare, M. R., Bird repellent., US Patent 2,967,128 (1961).
7. Glahn, J. F., Mason, J. R. & Woods, D. R., Dimethyl anthranilate as a bird repellent in livestock feed., *Wildl. Soc. Bull.*, **17** (1989) 313–20.
8. Cummings, J. L., Mason, J. R., Otis, D. L. & Heisterberg, J. F., Evaluation of dimethyl and methyl anthranilate as Canada goose repellent on grass. *Wildl. Soc. Bull.*, **19** (1991) 184–90.
9. Avery, M. L., Evaluation of methyl anthranilate as a bird repellent in fruit crops. *Proc. Vert. Pest Conf.*, **15** (1992) 130–3.
10. Dolbeer, R. A., Belant, J. L. & Clark, L. Methyl anthranilate formulations to repel birds from water at airports and food at landfields. *Proc. Great Plains Wildl. Damage Contr. Conf.*, **11** (1993) 42–53.
11. Askham, L. R., Efficacy of methyl anthranilate as a bird repellent on cherries, blueberries and grapes. *Proc. Vert. Pest Conf.*, **15** (1994) 137–41.
12. Mason, J. R. & Clark, L., Non-lethal repellents: the development of cost-effective practical solutions to agricultural and industrial problems. *Proc. Vert. Pest Conf.*, **15** (1992) 115–29.

13. Avery, M. L., Decker, D. G., Humphrey, J. S., Aronov, E., Lincombe, S. D. & Way, M. O., Methyl anthranilate as a rice seed treatment to deter birds. *J. Wildl. Manage.*, **59** (1995) 50–6.
14. Clark, L. & Shah, P., Non-lethal bird repellents: in search of a general model relating repellency and chemical structure. *J. Wildl. Manage.*, **55** (1991) 538–45.
15. Clark, L. & Shah, P., Chemical bird repellents: possible use in cyanide ponds. *J. Wildl. Manage.*, **57** (1993) 657–64.
16. Cummings, J. L., Pochop, P. A., Davis, J. E., Jr. & Krupa, H. W., Evaluation of ReJex-IT AG-36 as a Canada Goose grazing repellent. *Denver Wildl. Res. Cntr, Rep.*, **497** (1993).
17. Mason, J. R. & Clark, L., Evaluation of ReJex-IT AG-36 and activated charcoal as a snowgoose repellent. *Crop Protection*, **14** (1995) 467–9.
18. Mason, J. R., Adams, M. J. & Clark, L., Anthranilate repellency to starlings. Chemical correlates and sensory perception. *J. Wildl. Manage.*, **53** (1989) 55–64.
19. Clark, L., Mason, J. R. & Shah, P. Chemical repellency in birds: relationship between chemical structure and avoidance response. *J. Exp. Zool.*, **260** (1991) 310–22.
20. Clark, L. & Mason, J. R., Interaction between sensory and postingestional repellents in starlings: methyl anthranilate and sucrose. *Ecol. Applications*, **3** (1993) 434–59.
21. Belant, J. L., Gabrey, S. W., Dolbeer, R. A. & Seamans, T. W., Methyl anthranilate repels mallards and gulls from ponds. *Crop Protection*, **14** (1995) 171–5.
22. Henderson, S. T., *Daylight and its spectrum*. John Wiley, NY, 1977, pp. 102–3.
23. Malkin, J., *Photochemical and photophysical properties of aromatic compounds*. CRC Press, 1992, pp. 201–17.
24. Clark, L., Cummings, J., Bird, S. & Aronov, E., Acute toxicity of the bird repellent, methyl anthranilate, to fry of *Salmo salar*, *Oncorhinus mykiss*, *Ictalurus punctatus* and *Lepomis macrochirus*., *Pestic. Sci.*, **39** (1993) 313–17.